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From: Gabel, Gailene
Sent: Monday, February 14, 2000 8:51 AM
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Please obtain the following document:

- 1) Leclercq et al., Metabolism of very low density lipoproteins in genetically lean or fat lines of chicken, *Reproduction, Nutrition, Development*, 30 (6): 701-715 (1990)
- 2) Sato et al., Lipoprotein hydrolysis and fat accumulation in chicken adipose tissues are reduced by chronic administration of lipoprotein lipase monoclonal antibodies, *Poultry Science* 78 (9): 1286-1291 (1999).

Thank you!!!

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Please provide copy of the following literature:

1) Aoubala, et al., Epitope mapping and immunoactivation of human gastric lipase using five monoclonal antibodies, Eur J Biochem 211 (1-2): 99-104 (1993).

2) Aoubala et al., Immunological technique for the characterization of digestive lipases, Methods Enzymol 286 (Lipases part B), 126-149 (1997).

Thanks a bunch!

Gail Gabel
305-0807
7B15

Metabolism of very low density lipoproteins in genetically lean or fat lines of chicken

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Summary — Metabolism of very low density lipoproteins (VLDL) has been compared in fat (FL) and lean (LL) lines of chicken. When refed after fasting, plasma triglyceride concentration reached a significantly higher plateau in FL, although their feed consumption was lower than in LL. Newly synthesized VLDL were studied using anti-lipoprotein lipase antibodies. VLDL triglyceride (TG) concentrations were increased by antibody injection and reached a higher concentration in FL plasma than in LL. Newly synthesized VLDL exhibited a similar lipid composition. Fatty acid profiles were also similar when birds ingested a very low fat diet. Comparison of *in vitro* affinity of lipoprotein lipase and VLDL from both genotypes did not reveal any difference in K_m and V_{max} . [^{14}C] labelled VLDL from fat or lean donors were prepared and were injected into chickens from both genotypes. Fractional rate constants did not differ between lines. However, as plasma VLDL-TG pools were very different, plasma turnover was higher in FL than in LL. About 3-fold more VLDL-TG were incorporated in abdominal fat of FL than in LL. Difference in fattening between both genotypes seem to be due to both increased VLDL secretion and VLDL removal from plasma without difference in VLDL characteristics.

chicken / lipoprotein / adiposity / lipoprotein lipase / triglyceride

Résumé — Métabolisme des lipoprotéines de très basse densité (VLDL) chez les poulets génétiquement maigres ou gras. Lors d'une réalimentation, les poulets de la lignée grasse consomment moins rapidement leur aliment que ceux de la lignée maigre. Cependant, les triglycérides plasmatiques apparaissent en concentration plus élevée dans la lignée grasse. Des VLDL natives ont été obtenues après injection d'anticorps anti-lipoprotéine-lipase. La concentration en VLDL du plasma des poulets gras est alors significativement plus élevée que celle des maigres, signant une sécrétion hépatique plus intense. Dans ces conditions, la répartition des différentes classes de lipides est la même chez les 2 génotypes. Quand les animaux ingèrent un aliment très pauvre en acides gras aucune différence n'est observée dans la composition en acides gras de ces VLDL. Si le régime contient des acides gras non synthétisables par le poulet, les VLDL des poulets maigres sont plus riches en ces acides exogènes. Les constantes de cinétique enzymatique (K_m et V_{max}) des VLDL avec la lipoprotéine-lipase sont identiques chez les 2 génotypes. Enfin, des injections de VLDL marquées par le ^{14}C en provenance de poulets gras ou de poulets maigres ont été réalisées chez des receveurs de 2 lignées. Les constantes de renouvellement du pool plasmatique de VLDL ne sont pas différentes entre génotypes. Toutefois, du fait de pools plasmatiques de tailles très différentes, les quantités de VLDL sécrétées par le foie et captées par les tissus sont très supérieures chez les poulets gras. Quel que soit le génotype du donneur, les VLDL sont métabolisées de la même façon à l'intérieur de chaque génotype. Plus de 90% de la radioactivité est retrouvée dans les lipides du foie, du tissu adipeux abdominal et des autres lipides corporels. Cependant, 3 fois plus d'acides gras des VLDL sont incorporés dans le tissu adipeux abdominal des poulets gras que dans

celui des poulets maigres. Toutes ces observations suggèrent que: 1) la lignée grasse secrète plus de VLDL que la lignée maigre; 2) les VLDL des 2 lignées sont identiques et ne présentent aucune anomalie modifiant leur utilisation métabolique; 3) les poulets gras présentent la particularité de capter beaucoup plus d'acides gras des VLDL dans leur tissu adipeux abdominal.

poulet / lipoprotéine / adiposité / lipoprotéine- lipase / triglycérile

INTRODUCTION

To study mechanisms involved in excessive fat deposition of modern broiler chickens, a fat line (FL) and a lean line (LL) have been selected (Leclercq *et al*, 1980; Leclercq, 1988). No difference could be observed between lines for basal metabolic rate (Leclercq and Saadoun, 1982; Geraert *et al*, 1988b), thermogenesis (Geraert *et al*, 1988b) or feed intake (Leclercq and Saadoun, 1982; Geraert *et al*, 1988a). The main difference came from partitioning of energy between protein gain and lipid gain, lipid proportion always being significantly superior in FL chickens, even when feed intake was restricted (Leclercq and Saadoun, 1982). Since *de novo* fatty acid synthesis is low in avian adipose tissue (Leveillé *et al*, 1968) the difference in adiposity between FL and LL may be related to difference in availability in plasma triglyceride-rich lipoproteins such as very low density lipoproteins (VLDL) of hepatic origin. Indeed, fat chickens exhibited higher plasma VLDL concentration in the fasted and fed state (Hermier *et al*, 1984), together with an increase in hepatic lipogenesis (Saadoun and Leclercq, 1987). Moreover, hyper-VLDLemia found in FL could not be attributed to a defect in adipose tissue lipoprotein lipase (LPL) (Hermier *et al*, 1989). Nevertheless, since plasma VLDL consist of a mixture of native particles of hepatic origin and of particles which have been partially catabolized, the

direct relationship between adipose tissue deposition and VLDL secretion has still to be investigated. The present study was performed to address this question and to assess the rate of *in vivo* VLDL secretion in LL and FL chickens. Prevention of VLDL degradation by a specific inhibition of LPL was used to provide qualitative and quantitative characterization of native hepatic VLDL. Moreover, comparison of our experimental lines with those from Griffin *et al* (1989) for VLDL secretion and degradation can elucidate the origin of hyper-VLDLemia and adiposity in broiler chicken.

MATERIAL AND METHODS

Animals and diets

Male chickens from the tenth generation of FL and LL birds were used (Leclercq, 1988). They were housed collectively in floor pens (room temperature 25°C) until experiments started. Birds were fed low-fat diets, as mentioned in the *Results* section. This was done to minimize synthesis of intestinal portomicrons (analogous to mammalian chylomicrons) susceptible of interfering with VLDL metabolism, and to enhance *de novo* hepatic lipogenesis (Saadoun and Leclercq, 1987).

Preparation of anti-LPL antisera

Chickens adipose tissue (3 kg) was obtained from a commercial slaughterhouse and immedi-

ately frozen in liquid nitrogen. Adipose tissue was purified by affinity chromatography. The procedure was that described by Griffin *et al* (1976), except that adipose tissue was ground in distilled water (1 g tissue/ml water) to eliminate most of the lipid. Adipose tissue was then prepared. Affinity chromatography was performed on acetone powders was performed on the same eluant sequence (Griffin *et al*, 1976). LPL-containing tube was used for rapid lipolysis test. LPL so mixing those tubes and then drying was performed the day of the day. Antibodies against LPL were raised in sheep. LPL extracts were mixed with Freund's adjuvant and injected in the derma of 2 sheep. LPL was purified every 2 weeks. Blood samples were taken several times from 6 weeks after immunization. Immunoglobulin fraction was purified by precipitation in saturated ammonium sulfate solution. Precipitate was dialyzed against 0.1 M, dialyzed twice against 0.1 M ammonium sulfate, and stored at -20 °C.

In vitro inhibition of LPL by anti-LPL plasma. Two hundred microliters of adipose tissue extract (≈ 1.5 g adipose tissue/ml) were mixed with 100 ml heparin solution. Heparin plasma were mixed with 0.1 M-NH₄OH buffer (pH 7.4). Anti-LPL globulin fraction was added in different dilutions were corrected for protein steps (n from 1 to 7); the control was made without anti-LPL solution, giving the LPL activity at +4 °C, 10 μ l of rabbit anti-LPL were added. Thirty minutes later centrifuged for 5 min at 2000 g. The supernatant remaining LPL activity. According to the procedure of Hermier *et al*, 1989). Our anti-LPL was the same dilution steps best responder of the 2nd following experiments.

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ately frozen in liquid nitrogen. Chicken LPL was purified by affinity chromatography. The procedure was that described by Kompiang *et al* (1976), except that adipose tissue was directly ground in distilled water (without heparin) to eliminate most of the lipids; acetone powders were then prepared. Affinity chromatography of acetone powders was performed at + 3°C using the same eluant sequences as Kompiang *et al* (1976). LPL-containing tubes were selected by a rapid lipolysis test. LPL solution was made by mixing those tubes and then frozen. Freeze-drying was performed the day before immunization. Antibodies against chicken LPL were raised in sheep. LPL extract (500 µg protein) were mixed with Freund's adjuvant and injected in the derma of 2 sheep. Injections were made every 2 weeks. Blood samples were collected several times from 6 weeks after the first immunization. Immunoglobulin fraction was prepared by precipitation in saturated ammonium sulfate solution. Precipitate was dissolved in NaCl 0.15 M, dialyzed twice against NaCl 0.15 M to eliminate ammonium sulfate, and then frozen at -20 °C.

In vitro inhibition of LPL was performed using LPL from either adipose tissue or post-heparin plasma. Two hundred microliters of adipose tissue extract (= 1.5 g adipose tissue ground in 100 ml heparin solution (50 IU/ml)) or post-heparin plasma were mixed with 150 µl of NH₄Cl (0.1 M)-NH₄OH buffer (pH 8.6) and 100 µl of anti-LPL globulin fraction (pure or diluted). Different dilutions were compared using the 2ⁿ-steps (*n* from 1 to 7); the diluent was saline. A control was made without LPL-antibodies (saline solution), giving the LPL activity. After 30 min at +4 °C, 10 µl of rabbit anti-sheep whole serum were added. Thirty min later this mixture was centrifuged for 5 min at 2 000 g. Four hundred microliters of supernatant were used to measure remaining LPL activity. LPL was assayed according to the procedure described earlier (Hermier *et al*, 1989). Our antibodies were compared to those donated by Griffin *et al* (1989), using the same dilution steps. Antibodies from the best responder of the 2 sheep were then used in following experiments.

Experimental designs

Effect of refeeding on plasma triglycerides and VLDL composition

Six-week-old male chickens from the FL and LL were placed in individual cages and fed a low fat diet (22 g total fatty acids/kg feed). They were fasted overnight (18 h). They were refed *ad libitum* and individual feed consumptions were recorded. Blood samples were taken from wing vein 0, 30, 60, 90, 120 and 180 min after refeeding. Three groups of 6 birds were used per genotype; they were sampled at 0 and 90, 30 and 120 and 60 and 180 min respectively. Plasma were separated by low speed centrifugation and triglycerides (TG) determined. VLDL were characterized after centrifugation (see below).

Characterization of VLDL after *in vitro* inhibition of LPL

Five-week-old male chickens from the FL were used to determine the volume of anti-LPL globulin fraction to be injected in order to block completely chicken LPL. FL chickens were chosen since they were shown to have higher LPL activity (Hermier *et al*, 1989). In the first experiment, 3 ml of anti-LPL globulin fraction from the best responder sheep were injected intravenously in 3 chickens. Birds were sampled (1 ml blood) at wing vein every 15 min after immunization until 120 min.

In the second experiment 1, 2 or 3 ml of anti-LPL globulin were injected in 4 chickens per treatment. Blood samples were collected 1 h after passive immunization. Plasma triglyceride concentrations were measured according an enzymatic method (see below).

In the following experiments it was decided from the 2 previous experiments to inject 1.5 ml of anti-LPL globulin fraction per kg live weight and to collect blood 1 h later. In one experiment comparison of TG increase due to LPL inhibition was performed using FL and LL 5-wk-old male chickens (10 birds per line) and adult (24 wk of

age) cockerels (8 birds per line). Similar number of birds were used as controls; they were injected with plasma globulin fraction from non-immunized sheep. Ten ml of blood were collected using EDTA as anticoagulant and centrifuged; plasma aliquots were kept for analysis of VLDL composition and fatty acid composition of VLDL-TG. In all these experiments birds were given a low-fat diet (22 g total fatty acids/kg feed) containing maize (300 g/kg), wheat (280 g/kg), soybean meal (280 g/kg), minerals and vitamins.

Another experiment was undertaken to determine the fatty acid composition of VLDL-TG from 5-wk-old male chickens fed a very low-fat diet (2.4 g total fatty acids/kg feed) in order to minimize the influence of exogenous dietary fatty acids. Birds were fasted overnight (18 h) and then force-fed a mixture containing water (400 g/kg), corn starch (300 g/kg), soybean proteins (150 g/kg), straw (80 g/kg), minerals and vitamins. Birds were injected with anti-LPL-globulin fraction 1 h after tubing. Blood sampling was performed 1 h later.

Turnover of plasma VLDL-TG

Endogenously labelled VLDL were prepared using either FL or LL donors. In the first experiment, 2 FL donors (≈ 1.5 kg live weight) were used. Thirty-seven MBq (999 μ Ci) of [14 C]-1-palmitate (specific activity 8.5 MBq/mg) were dissolved in 1 ml ethanol + 70 μ l 1% NaOH-ethanol and kept 4 h at ambient temperature. Ethanol was then evaporated and sodium palmitate was mixed with 5 ml chicken plasma prepared using EDTA as anticoagulant. Donors were force-fed a very low fat diet (2.4 g total fatty acids/kg diet) made from corn starch and soybean proteins to suppress the synthesis of intestinal portomicrons. One h later they were intravenously injected with 2.5 ml of the [14 C]-palmitate solution. Anti-LPL globulin fraction was intravenously injected 10 min later. About 50 ml of blood were collected 1 h later using EDTA as anticoagulant. VLDL were separated by ultracentrifugation (see *Analytical procedures*). VLDL were dialyzed against NaCl 0.15 M to eliminate EDTA. Aliquots were kept to determine chemical composition and distribution of radioactivity. Labelled VLDL (1 ml, 4,381,000 dpm) were intravenously injected in FL and LL 7-wk-old male chickens (8 per genotype). Birds of similar live weight were kept in individual

cages and fed the low fat diet (22 g total fatty acids/kg feed) (see above). Five ml of blood were collected 10, 20 and 30 min after injecting labelled VLDL. Birds were immediately slaughtered by decapitation. Abdominal fat, liver and remaining carcass were weighed, frozen and kept for further analysis. A similar experiment was performed using LL donors, except that only 1,841,000 dpm of labelled VLDL were injected per bird (8 per genotype). Plasma volume was estimated at different ages (28, 42, 56, 70 d and in adults) by the Evans blue method. Half a ml of Evans blue solution (1% in saline) was intravenously injected per kg live weight. Dilution volume (plasma volume) was estimated from dye concentration of plasma.

Kinetic properties of LPL compared to VLDL from LL and FL chickens

VLDL from FL and LL chickens were compared for their enzymatic characteristics to LPL. LPL solution was prepared by grinding adipose tissue from commercial cross-broiler following the procedure used to measure *in vitro* LPL inhibition (see above). VLDL were prepared as described above using anti-LPL antibodies. A first experiment was performed to determine the time for measuring initial rate of hydrolysis (V_0). Three triglyceride concentrations (0.156, 0.625 and 2.5 mg/ml) were compared after 5, 10, 15, 20 min of enzymatic reaction. Triglyceride substrate (0.5 ml) was mixed with 0.5 ml of a buffer solution containing bovine serum albumin (BSA) (BSA: 60 g/l, Tris buffer: 50 mM, CaCl_2 : 5 mM, pH 8.6) and 1 ml of LPL solution (4 g chicken adipose tissue from a commercial cross, ground in 100 ml heparin solution). Reaction was stopped by adding 5 ml of isopropanol-heptane (4 V/1 V). Since concentration of liberated non esterified fatty acid (NEFA) (see below for determination) was found linear according to time only until 10 min, the following experiment was performed at 10 min. Michaelis-Menten constants (K_m and V_{max} of hydrolysis of LL- and FL-VLDL by LPL were estimated and compared to Intralipid substrate usually selected to measure LPL activity. Intralipid was activated by chicken serum (v/v). Nine final concentrations of medium triglycerides were compared (see legend to table VII) for VLDL substrates. Supplementary concentrations were used for Intralipid since TG hydrolysis of this artificial substrate was substantially lower than that of VLDL. K_m and V_{max} were estimated from Lineweaver-Burk plots.

Analytical procedures

Blood samples were collected with EDTA as anticoagulant, and plasma was separated by centrifugation for 20 min at +4 °C. Labeled and non-labelled VLDL from plasma by ultracentrifugation at +10 °C for 17 h (Hermi a Beckman ultracentrifuge) and were then dialyzed against distilled water to eliminate EDTA, especially destined for reinjection in study of kinetic properties of

The following components were measured in plasma or in VLDL: protein by the Lowry method (1951) using a standard, triglycerides, phospholipids and total cholesterol using the method of Folch (1956) modified by Bio-Mérieux (Charleville, France). Cholesteryl esters were measured using the formula:

cholesteryl esters : (total cholesterol) $\times 1.67$

NEFA was measured by the method of Fruchart *et al* (1974). Lipids were extracted from carcasses, plasma and tissues following the Folch method. Fatty acid composition was determined by gas-liquid chromatography using a GC 180, GIRA, France) on a 2.5 m liquid phase.

VLDL turnover was studied by liquid scintillation counting (carb-460 CD). Specific activity of VLDL-TG were plotted against time. Non-linearity test was performed in each experiment; if tests were never significant, it was assumed that data could be analysed as a linear model. Amounts of VLDL-TG in tissues and organs were determined by dividing total incorporated radioactivity by the specific activity of plasma given by the following equation:

$$A = \frac{1}{30} A_0 \int_0^t$$

diet (22 g total fatty acid). Five ml of blood were taken immediately after slaughtering the birds, and the abdominal fat, liver and weighed, frozen and stored at -20 °C. A similar experiment was performed with 10 donors, except that the labelled VLDL were injected intravenously (type). Plasma volume was estimated by the dye dilution method (28, 42, 56, 70 d). Half a (1% in saline) was injected intravenously. Dilution was estimated from the radioactivity of the plasma.

LPL compared to chicken

Chickens were compared to LPL. LPL was prepared by grinding adipose tissue from broiler following the procedure in *vitro* LPL inhibition assay. LPL were prepared as described by LPL antibodies. A first experiment was performed to determine the rate of hydrolysis (V_0) of triglycerides (0.156, 0.625, 1.25, 2.5, 5, 10, 15, 30, 60, 120, 240, 480, 960, 1920, 3840, 7680, 15360, 30720, 61440, 122880, 245760, 491520, 983040, 1966080, 3932160, 7864320, 15728640, 31457280, 62914560, 125829120, 251658240, 503316480, 1006632960, 2013265920, 4026531840, 8053063680, 16106127360, 32212254720, 64424509440, 128849018880, 257698037760, 515396075520, 1030792151040, 2061584302080, 4123168604160, 8246337208320, 16492674416640, 32985348833280, 65970697666560, 131941395333120, 263882790666240, 527765581332480, 1055531162664960, 2111062325329920, 4222124650659840, 8444249301319680, 16888498602639360, 33776997205278720, 67553994410557440, 135107988821114880, 270215977642229760, 540431955284459520, 1080863910568919040, 2161727821137838080, 4323455642275676160, 8646911284551352320, 17293822569102704640, 34587645138205409280, 69175290276410818560, 138350580552821637120, 276701161105643274240, 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tion of 487.7 mg palmitic acid/h; *ie*, on average, the total LPL activity from 50 g chicken adipose tissue (abdominal fat). When using post-heparin plasma, 80–90% of triglyceride hydrolytic activity was inhib-

ed by anti-LPL globulin fraction. As a control, a similar inhibitory effect was obtained using protamine sulfate as LPL inhibitor. Under the same conditions, anti-LPL fraction was twice as potent in inhibiting LPL than that of Griffin *et al* (1989; not shown).

As shown in figure 2, injection of anti-LPL globulins into fed chickens induced a sharp increase in plasma VLDL-TG concentrations, whereas no change was observed in chickens injected with non-immunized sheep serum. When using 3 ml of anti-LPL globulin fraction, increase of plasma VLDL-TG plateaued 90 min after injection, reaching a concentration increment of 5 g/l. Thus plasma TG concentration was multiplied by ≈ 7 after 90 min, which is slightly superior to the figures observed by Kompang *et al* (1976). Three doses (1, 2 and 3 ml) of anti-LPL globulins were compared in the second experiment. Plasma triglyceride concentrations were respectively 6.00 ± 0.56 , 7.31 ± 0.69 and 6.35 ± 0.81 g/l 1 h after injection; there was no significant difference between doses. In the following experiments 1.5 ml globulin fraction/kg live weight and 60 min delay were chosen to reach maximum VLDL increase.

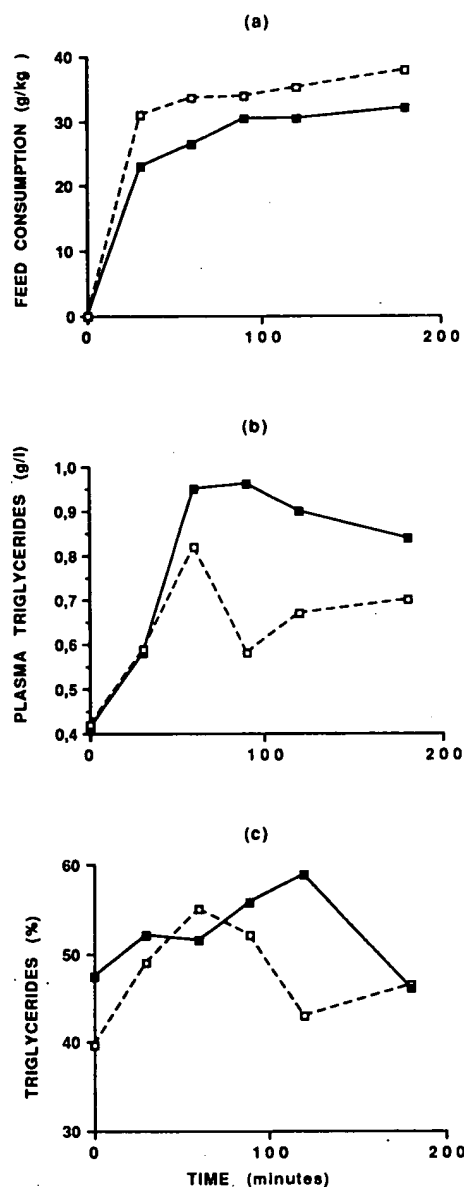


Fig 1. Pattern of feed consumption (a), plasma triglyceride concentration (b) and triglyceride percentage in VLDL (c), in genetically lean and fat chickens during refeeding. Lean (□) and fat (■) 6-wk-old male chickens were fasted overnight (18 h) and then were fed a low-fat diet (22 g lipid per kg diet). Mean live weights were 1348 ± 23.4 g and 1251 ± 28.4 g for LL and FL respectively. Analysis of variance using genotype and time as factors led to significant effect of genotypes on plasma TG ($P < 0.01$) and TG percentage in VLDL ($P < 0.05$). Differences for feed consumption (g feed per kg live weight) were only significant 30 and 60 min after refeeding.

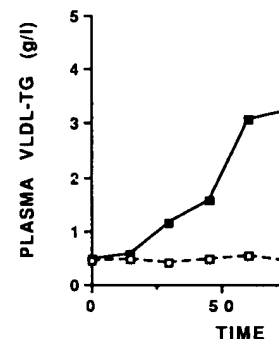


Fig 2. Plasma VLDL-TG of fat chicken after intravenously injected anti-LPL antibodies. Fat chickens (3 birds) were injected with anti-LPL globulin fraction (■). Controls were injected with 3 ml globulin fraction from non-immunized sheep (□).

Secretion rate and composition of newly synthesized VLDL

Secretion rate of plasma triglycerides in FL and LL chickens 35 d of age or at the time of laying are given in figure 3. Plasma triglycerides due to LPL are always superior in FL to LL. The triglyceride increase was always superior in FL to LL in adult cockerels.

The composition of VLDL in FL and LL chicken is given in table II. In the control group (without LPL blockade), no large difference was observed between genotypes, the triglyceride percentage in VLDL being superior to the triglyceride percentage in plasma. In contrast, newly synthesized VLDL LPL blockade exhibited a large difference in both genotypes.

Fatty acid composition is given in table II. Two experiments were performed: one with a low-fat diet (22 g lipid/kg feed) and one with a diet containing 2 g lipid/kg feed.

fraction. As a consequence was obtained as LPL inhibitor. Injections, anti-LPL fraction in inhibiting LPL (1989; not shown).

2, injection of anti-chickens induced a plasma VLDL-TG concentration change was observed 90 min after injection with non-immune. When using 3 ml globulin fraction, increase of plasma TG concentration was observed 90 min after injection, or to the figures obtained *et al* (1976). Three of anti-LPL globulins were used in second experiment. Concentrations were 5.6, 7.31 \pm 0.69 and 1.5 ml globulin fraction; there was no difference between doses. In experiment 1.5 ml globulin fraction and 60 min delay after injection maximum VLDL in-

consumption (a), plasma triglyceride (b) and triglyceride in genetically lean and fat chickens. Lean (\square) and fat (\blacksquare) chickens were fasted overnight and then refed a low-fat diet. Mean live weights were 729 \pm 28.4 g for LL and FL respectively. Variance using genotypes had no significant effect on TG ($P < 0.01$) and TG ($P < 0.05$). Differences for TG per kg live weight) and 60 min after refeed-

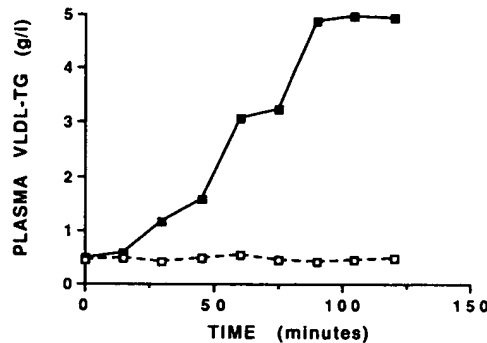


Fig 2. Plasma VLDL-TG concentration genetically fat chicken after intravenous injection of anti-LPL antibodies. Fed 5-wk-old male chickens (3 birds) were injected with 3 ml anti-LPL-globulin fraction (\blacksquare). Control group (3 birds) were injected 3 ml globulin fraction from non-immunized sheep (\square).

Secretion rate and composition of newly synthesized VLDL

Secretion rate of plasma triglyceride was compared in FL and LL male chickens at 35 d of age or at the adult state. Results are given in figure 3. Increase in plasma triglycerides due to LPL inhibition was always superior in FL to that of LL. However, triglyceride increase was much lower in adult cockerels.

The composition of VLDL from 5-wk-old FL and LL chicken is given in table I. In the control group (without LPL blockade), a large difference was observed between genotypes, the triglyceride content of FL-VLDL being superior to that of LL. In contrast, newly synthesized VLDL obtained by LPL blockade exhibited a similar composition in both genotypes.

Fatty acid composition of VLDL-TG is given in table II. Two experiments were performed: one with a diet containing 22 g total fatty acids/kg feed (exp 1), the other with a diet containing 2.4 g total fatty acids/kg

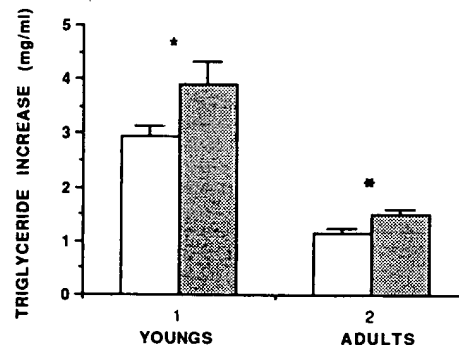


Fig 3. Increase of plasma triglyceride concentration induced in genetically lean (\square) and fat (\blacksquare) chickens 1 h after intravenous injection of anti-LPL antibodies. Mean live weights of 5-wk-old male chickens were 729 g and 745 g for LL and FL respectively. Mean weights of adult cockerels were 3 794 g and 3 384 g for LL and FL respectively. All birds were fed *ad libitum* and injected with 1.5 ml anti-LPL-globulin fraction per kg live weight. (*) = Difference significant at the 0.05 level.

kg feed (exp 2). In experiment 1, LL chickens exhibited significantly higher proportions of linoleic (18:2) and linolenic (18:3) acids. Very similar concentrations were found for the other fatty acids. When fed a diet almost devoid of fatty acids, no difference was observed between lines for fatty acid composition.

Comparison of VLDL-TG turnover in fat and lean chickens

Two experiments were undertaken to study the plasma VLDL-TG turnover. Fat donors were used in the first experiment and lean donors in the second experiment. In both experiments, regressions were calculated between time and logarithms of specific activity of VLDL-TG. Non-linearity of relationship between both variables was tested for each line by the Fisher-test (F).

Table I. Composition (%) of VLDL of plasma from lean (LL) or fat (FL) 5 week-old chickens injected anti-LPL antibodies or control globulin fraction.

	n	Free cholesterol	Cholesterol esters	Triglycerides	Phospholipids	Proteins
Control globulin fraction						
LL	7	3.5 ± 0.3 ^a	9.8 ± 0.9 ^b	62.6 ± 2.0 ^a	15.1 ± 1.1 ^b	9.1 ± 0.4 ^b
FL	8	3.1 ± 0.2 ^a	6.8 ± 0.6 ^a	68.4 ± 1.6 ^b	13.1 ± 0.3 ^a	8.9 ± 0.5 ^b
Anti LPL globulin fraction						
LL	10	4.6 ± 0.1 ^b	5.9 ± 0.5 ^a	68.7 ± 1.1 ^b	13.5 ± 0.3 ^a	7.3 ± 0.4 ^a
FL	12	4.2 ± 0.1 ^b	6.0 ± 0.7 ^a	68.5 ± 1.4 ^b	13.4 ± 0.3 ^a	7.8 ± 0.5 ^a

* Means ± standard deviation of mean from *n* data per treatment. Means with the same letter are not different at the 0.05 level.

In experiment 1, *F*-values were 0.48 and 2.36 (degrees of freedom 1 and 21) for FL and LL respectively. Corresponding *F* values were 0.40 and 0.25 in the second experiment. As non-linearity tests were not significant, regressions were considered

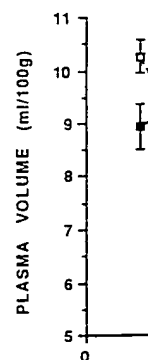
as linear and thus it was assumed that decreasing activity from injection to 30 min may be analyzed as a one-compartment model. These observations confirm earlier conclusions by Kudzman *et al* (1975) in chicken.

Table II. Fatty acid composition of plasma VLDL-TG from genetically lean (LL) or fat (FL) chickens injected anti-LPL antibodies.

Total fatty acid content of diet (g/kg)	Genotype	LL n	Exp 1 22.0 ¹	FL 10	Exp 2 2.4	LL 7	FL 7
14:0			0.8 ± 0.1 NS	1.0 ± 0.1		0.9 ± 0.1 NS	1.0 ± 0.1
16:0			31.4 ± 1.4 NS	34.2 ± 1.3		30.3 ± 1.2 NS	32.8 ± 0.9
16:1			4.1 ± 0.2 NS	5.1 ± 0.6		6.3 ± 0.7 NS	5.3 ± 0.2
18:0			12.1 ± 0.7 NS	12.5 ± 0.7		10.5 ± 0.9 NS	11.5 ± 0.5
18:1 _{n-9}			31.4 ± 1.4 NS	30.8 ± 1.4		43.6 ± 1.4 NS	41.1 ± 1.5
18:2 _{n-6}			18.9 ± 1.4 ^{**}	15.6 ± 1.0		8.2 ± 1.8 NS	7.9 ± 1.4
18:3 _{n-3}			1.2 ± 0.2 [*]	0.8 ± 0.1		0.3 ± 0.04 NS	0.4 ± 0.1

NS = Non significant difference; * = difference significant at the 0.05 level; ** = difference significant at the 0.01 level. ¹ Respective proportion of linoleic (18:2) and linolenic (18:3) acids in this diet were: 55.3 and 3.6% of fatty acids.

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**Fig 4.** Plasma: genetically lean to age. Lean between 6 and 10 weeks and then in containing 1 weight). Blood later. Two-wa cant effect of from 28, 42, 5

Results from the first experiment are presented in tables III and IV. As expected, FL chickens were fatter than LL. VLDL-TG concentrations were higher in plasma of FL than in LL. In order to estimate the total VLDL-TG plasma pool, the plasma volume was measured in both lines at different ages (fig 4). The plasma volume of FL was lower than that of LL during the growing period (28, 42, 56 and 70 d of age), when expressed as ml/100 g live weight. However, the total plasma VLDL-TG pool was significantly larger in FL than in LL (table III). Fractional rate constants were very similar in both genotypes; but the turnover of plasma VLDL-TG was significantly more important in the fat genotype. Thirty minutes after the injection of labelled VLDL, most of the radioactivity was found in extraplasmaic compartment (adipose tissues and organ lipids): recoveries were 93.9% and 96.7% for LL and FL respectively. A significantly higher percent-

age of radioactivity was recovered in abdominal fat of FL. VLDL-TG uptake was more pronounced in abdominal fat and extra abdominal lipids of the fat genotype; however, this was significant only for abdominal fat.

Results from the second experiment using lean donors are given in tables V and VI. Although slightly different in absolute values, results are very similar when comparison is made between genotypes.

Enzymatic characteristics of LPL against VLDL from fat and lean chickens

K_m and V_{max} values of LPL against VLDL from LL and FL, and Intralipid are presented in table VII. Since slopes and constants from regression lines in Lineweaver-Burk plots were not different between the VLDL in the 2 lines, we may conclude that both K_m and V_{max} do not differ between LL and FL chickens. When Intralipid (activated by equal volume of chicken serum) was used as substrate, a similar V_{max} was observed, whereas K_m was significantly higher than that obtained with VLDL (at least 2 orders of magnitude).

DISCUSSION

Differences in the plasma concentrations of triglycerides and VLDL have been found between these genotypes in several circumstances (Hermier *et al*, 1984). This was confirmed again in the present results. Plasma TG reached a higher plateau in FL than in LL after refeeding; this was found despite a lower feed consumption rate in fat chickens. This discrepancy between feed ingestion and TG increase in plasma of both genotypes reinforces our previous

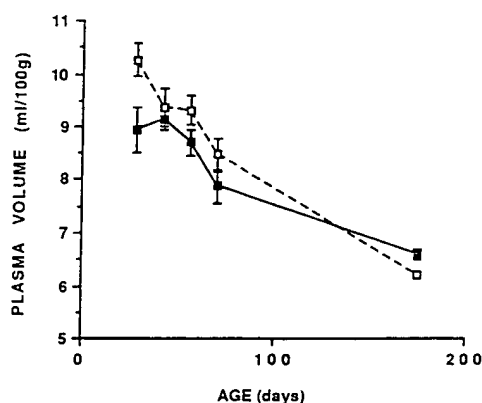


Fig 4. Plasma volume (ml/100 g live weight) of genetically lean and fat male chickens according to age. Lean (□) and fat (■) male chickens (between 6 and 10 per age) were fasted overnight and then injected with physiological saline containing 1% Evans blue (0.5 ml/kg live weight). Blood samples were collected 5 min later. Two-way analysis of variance led to significant effect of genotype ($F_{43} = 9.6$) when data from 28, 42, 56 and 70 d of age were used.

chickens injected

ids Proteins

2 9.1 ± 0.4^b
3 8.9 ± 0.5^b

3 7.3 ± 0.4^a
3 7.8 ± 0.5^a

are not different at the

assumed that de-
duction to 30 min
one-compartment
s confirm earlier
et al (1975) in

at (FL) chickens in-

2

FL
7

1.0 ± 0.1
32.8 ± 0.9
5.3 ± 0.2
11.5 ± 0.5
41.1 ± 1.5
7.9 ± 1.4
0.4 ± 0.1

significant at the 0.01
5.3 and 3.6% of fatty

Table III. Live weight, carcass lipids and VLDL-TG metabolism of lean (LL) and fat (FL) 7 week-old male chickens injected C¹⁴-labelled VLDL from fat donors.

	Live weight (g)	Abdominal fat lipids (g)	Extra- abdominal lipids(g)	Plasma VLDL-TG (mg/ml)	Plasma VLDL-TG pool (mg)	VLDL-TG fractional rate constant (min ⁻¹)	VLDL-TG turnover (mg/min)
LL	1517 ± 52.6	8.75 ± 1.83	142.4 ± 11.1	0.471 ± 0.044	66.10 ± 5.82	-0.1008 ± 0.0033	6.57 ± 0.45
FL	1658 ± 46.2	43.51 ± 3.78	201.6 ± 9.6	0.749 ± 0.055	110.8 ± 9.06	-0.1007 ± 0.0028	11.05 ± 0.74
<i>t</i>	1.99	8.61	3.97	3.97	4.25	0.02	5.31
NS	(<i>P</i> < 0.01)	(<i>P</i> < 0.01)	(<i>P</i> < 0.01)	(<i>P</i> < 0.01)	(<i>P</i> < 0.01)	NS	(<i>P</i> < 0.01)

* Means ± standard deviations of mean from 8 chickens; VLDL from donors contained 3.04 mg triglyceride per ml. Specific activity was 4 381 000 dpm/ml; 98% of radioactivity was in triglyceride fraction. VLDL composition was: 67.4% triglycerides, 8.6% total cholesterol, 12.9% phospholipids and 11.1% proteins.

Table IV. Radioactivity (FL) 7 week-old male

	Liver	Ab
LL	24.2 ± 0.54	5
FL	24.0 ± 1.96	1:
<i>t</i>	0.09	
	NS	(

conclusions that differed due to different feeding regimes. In the present study, the maximum deviation of the control (Simon and Leclercq *et al*, 1988; Th maximum concentration of VLDL was reached in the fat genotype (fig 1c), suggesting a longer time of VLDL secretion in the fat genotype.

Inhibition of LPL by antibodies allowed characterization of hepatic VLDL. The results appeared quantitative and different from VLDL from 5 wk of age plasma. The maximum concentration was multiplied by both genotypes (3.1 and 3.2 respectively) 60 min after injection. VLDL-TG secretion in the fat genotype was higher than in the lean one. Previous studies (Leclercq, 1988; *in vivo* fatty acid synthesis) showed a significantly higher rate of increased hepatic lipogenesis in the fat genotype, which was demonstrated by an increase in the

Table IV. Radioactivity distribution and amounts of VLDL-TG incorporated in lipids of lean (LL) and fat (FL) 7 week-old male chickens 30 min after injection of C¹⁴-labelled VLDL from fat donors.

	Radioactivity distribution (% of injected)			Recovery (%)	VLDL-TG incorporated (mg/30 min)	
	Liver	Abdominal fat	Extra-abdominal fat		Abdominal fat	Extra-abdominal fat
LL	24.2 ± 0.54	5.38 ± 0.70	64.3 ± 3.10	93.9 ± 2.91	15.01 ± 2.82	176.6 ± 23.6
FL	24.0 ± 1.96	12.7 ± 0.69	60.9 ± 1.63	96.7 ± 2.35	40.11 ± 5.81	189.2 ± 21.9
<i>t</i>	0.09	7.45	0.93	0.99	4.11	0.40
	NS	(<i>P</i> < 0.01)	NS	NS	(<i>P</i> < 0.01)	NS

conclusions that difference in fatness is not due to different feed intakes but to a metabolic deviation depending on hormonal control (Simon and Leclercq, 1985; Leclercq *et al*, 1988; Saadoun *et al*, 1988). The maximum concentration of TG in plasma VLDL was reached later in FL than in LL (fig 1c), suggesting that the prominence of VLDL secretion over their catabolism is longer in the fat chickens.

Inhibition of LPL activity by specific antibodies allowed characterization of native hepatic VLDL. These lipoproteins appeared quantitatively and qualitatively different from VLDL found in fed chickens. At 5 wk of age plasma triglyceride concentration was multiplied by a similar factor in both genotypes (3.7 and 3.5 in LL and FL respectively) 60 min following LPL inhibition. VLDL-TG secretion was 40% higher in the fat genotype as compared to the lean one. Previous experiments (Saadoun and Leclercq, 1987) have shown that *de novo* fatty acid synthesis in the liver is significantly higher in FL than in LL. This increased hepatic lipogenesis is thus accompanied by an increased VLDL secretion which was demonstrated by both LPL inhi-

bition and measurement of labelled VLDL turnover.

As previously observed (Hermier *et al*, 1989), the proportion of triglyceride-rich particles appears to be higher as a consequence of their increased secretion rate. On the contrary, newly secreted VLDL obtained by LPL-blockade exhibited similar composition and lipid distribution in both genotypes. Their fatty acid compositions were different only when birds ingested a diet containing fat (even at low level on inclusion). Under these conditions, exogenous fatty acids, such as linoleic (18:2 *n*-6) and linolenic (18:3 *n*-3) acids, which cannot be synthesized by chicken, were found at significantly higher concentrations in LL than in FL. This observation confirms a similar conclusion made by Legrand and Lemarchal (1987) and Nitsan *et al* (1986). This may be due to the lower *de novo* fatty acid synthesis in liver of LL chickens, which leads to a less pronounced dilution of exogenous fatty acids by endogenous fatty acids. When diet was practically devoid of fatty acids (2.4 g fatty acids per kg diet) no difference was noticed.

Table V. Live weight, carcass lipids and VLDL-TG metabolism of lean (LL) and fat (FL) 7-week-old male chickens injected ^{14}C -labelled VLDL from lean donors.

	Live weight (g)	Abdominal fat lipids (g)	Extra- abdominal lipids (g)	Plasma VLDL-TG (mg/ml)	Plasma VLDL-TG pool (mg)	VLDL-TG fractional rate constant (min^{-1})	VLDL-TG turnover (mg/min)
LL	1464* \pm 68.2	14.54 \pm 3.02	163.1 \pm 20.5	0.312 \pm 0.051	41.74 \pm 6.53	-0.0889 \pm 0.0151	3.16 \pm 0.44
FL	1528 \pm 72.2	51.36 \pm 3.14	242.9 \pm 20.6	0.601 \pm 0.063	81.73 \pm 9.62	-0.0736 \pm 0.0020	6.64 \pm 1.14
<i>t</i>	0.60 NS	8.43 ($P < 0.01$)	2.73 ($P < 0.05$)	3.59 ($P < 0.01$)	3.52 ($P < 0.01$)	0.94 NS	2.98 ($P < 0.01$)

* Means \pm standard deviations of mean; VLDL from donors contained 3.08 mg triglyceride per ml. Specific activity was 1 841 200 dpm/ml; 96% of radioactivity was in tri-
glyceride fraction. VLDL composition was: 67.0% triglycerides, 7.2% total cholesterol, 13.0% phospholipids and 12.8% proteins.

Table VI. Radioac-
(FL) 7 week-old m

Liver	
LL	22.5 \pm 1.25
FL	23.8 \pm 1.55
<i>t</i>	0.68 NS

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Table VII. Enzym
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K_m (mg/ml)	V_{max}
($\mu\text{g NEFA}/10 \text{ min}$)	

* Means with the :
0.05 level. Initial r
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Table VI. Radioactivity distribution and amounts of VLDL-TG incorporated in lipids of lean (LL) and fat (FL) 7 week-old male chickens 30 min after injection of ^{14}C -labelled VLDL from lean donors.

	Radioactivity distribution (% of injected)			Recovery (%)	VLDL-TG incorporated (mg/30 min)	
	Liver	Abdominal fat	Extra-abdominal fat		Abdominal fat	Extra-abdominal fat
LL	22.5 \pm 1.25	5.74 \pm 0.69	62.6 \pm 1.95	90.8 \pm 2.01	10.95 \pm 2.42	116.3 \pm 19.9
FL	23.8 \pm 1.55	12.6 \pm 0.77	58.7 \pm 2.80	95.1 \pm 3.13	37.7 \pm 3.83	170.1 \pm 21.7
<i>t</i>	0.68	6.6	1.17	1.18	5.62	1.83
	NS	(<i>P</i> < 0.01)	NS	NS	(<i>P</i> < 0.01)	NS

Native VLDL obtained after LPL-inhibition have been used as a tool to investigate the *in vivo* metabolism of VLDL in LL and FL. Comparison of VLDL turnover showed that VLDL-TG incorporation is higher in adipose tissues of FL. This was true whatever the genotype of donors, suggesting that VLDL of FL and LL have the same ability to be withdrawn by adipose tissues. This conclusion is also suggested

Table VII. Enzymatic properties of chicken LPL against different triglyceride substrates.

	FL-VLDL	LL-VLDL	Intralipid
K_m (mg/ml)	0.2600 ^a	0.2430 ^a	32.86 ^b
V_{max} ($\mu\text{g NEFA}/10 \text{ min}$)	651.9	605.3	689.7

* Means with the same letter are not different at the 0.05 level. Initial reaction rates were measured after 10 minutes. Initial triglyceride concentration of medium were: 0.18, 0.37, 0.56, 0.75, 1.12, 1.5, 2.25, 3.0 and 3.75 mg/ml for VLDL substrate and 0.75, 1.12, 1.5, 2.25, 3.0, 3.75, 5.25, 6.75, 9.0 and 15 mg/ml for Intralipid. K_m and V_{max} were estimated by Lineweaver-Burk plot. Three replicates were used per substrate concentration.

by *in vitro* enzymatic characteristics of LPL against VLDL from both genotypes; different affinity between LPL and triglyceride substrates such as that observed between VLDL from the immature and laying hen (Bacon *et al*, 1978; Griffin *et al*, 1982) does not seem to exist between VLDL from FL and LL. Since FL possess twice the mature adipocytes of LL in abdominal fat (Hermier *et al*, 1989) and since uptake is more than doubled in the FL as compared to the lean genotype, we may assume that FL adipocytes are able to incorporate a little more VLDL-fatty acids than those from LL. Genetic observations suggest that difference in fatness between our fat and lean lines of chickens depends on a polygenic control (Leclercq, 1988). From our present investigations it is obvious that higher *de novo* fatty acid synthesis in FL chickens (Saadoun and Leclercq, 1987) is accompanied by an increase in VLDL secretion. The part of genes controlling fatness appear to act on hepatic synthesis and secretion of VLDL. However, VLDL composition, affinity for LPL and ability to be incorporated in adipose tissues appear to be very similar between genotypes. Moreover, VLDL-fatty acids are removed more intensively from the bloodstream in

FL. In this genotype the balance between higher VLDL secretion and removal leads to higher plasma VLDL and TG-concentrations, which cannot be due to a defect in VLDL catabolism.

The present observations can be compared to those obtained using other experimental chicken lines which were selected for high or low plasma VLDL-concentration by Griffin *et al* (1989). Differences in plasma VLDL concentrations are less pronounced between our 2 fat and lean lines than between lines selected on plasma VLDL, although difference in adiposity is more important between our lines. Both our fat line and the high VLDL-line from Griffin exhibit higher VLDL secretion than their lean counterpart. The VLDL-TG fractional rate constants were very similar in our lines, whereas they were significantly different in the lines selected on plasma VLDL-concentration, the low VLDL-line exhibiting a faster clearance of VLDL from the circulation than the high VLDL-line. This difference may be explained by differences in LPL activity of adipose tissues and other organs. Indeed in our lines total LPL activities per abdominal fat pad were very different, the FL having a higher LPL content, which is due to a higher number of adipocytes (Hermier *et al*, 1989). On the contrary, total LPL content of abdominal fat pad was not different between high and low VLDL lines; but in the low VLDL line higher LPL activity was found in heart and muscles, leading to preferential use of VLDL-TG by these organs.

In conclusion, genetic controls of adiposity and plasma VLDL in chicken may depend on several mechanisms such as VLDL secretion, proliferation of adipocytes, enzymatic equipment of adipose tissues and other organs. Some of these mechanisms may be independently distributed amongst genotypes.

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